Amendments to the Drawings

Figures 1 and 2 are amended to include the label "Prior Art."

A replacement Figure 5 is included herewith.

REMARKS

Reconsideration and withdrawal of the rejections set forth in the Office action dated September 19, 2005 are respectfully requested. Applicants petition the Commissioner for a 2-month extension of time. A separate petition accompanies this amendment.

I. Amendments

Claims 8-9 and 11-25 are canceled.

Claim 1 is amended to recite the lipid bilayer expanses have a component associated with the lipid bilayer expanse. Basis for this amendment can be found on page 6, lines 26-31. Claim 1 is further amended to clarify the interaction between the test agent and the lipid bilayer-associated component. Basis for this amendment can be found on page 6, lines 19-25 and 32-33. Finally, claim 1 is amended to recite the physical property is membrane fluidity. Basis for this amendment can be found in original claim 9.

Claims 2-4 are amended for consistent terminology.

Claim 10 is amended to depend from claim 1. Claim 10 is further amended to improve readability.

Claim 26 is amended for clarification of the steps.

Claims 27-29 are amended for clarity and for consistent terminology.

Claim 40 is amended to correct a typographical error as well as to improve readability.

Figures 1 and 2 are amended to include the label "Prior Art."

No new matter is added by way of these amendments.

II. Objection to the Oath/Declaration

Applicants will forward a Declaration executed in accord with 37 C.F.R. § 1.63 by the inventors under separate cover. Applicants respectfully request

withdrawal of the objection to the Oath/Declaration when such a Declaration is received.

III. Objection to the Drawings

The Examiner states that Figures 1 and 3 should be designated by a legend such as "Prior Art." Figure 5 is objected to as too dark. Applicants enclose herewith Replacement Figures 1 and 3 labeled "Prior Art" as suggested. Applicants further enclose herewith a replacement Figure 5. Accordingly, Applicants respectfully request withdrawal of the objections to the Drawings.

IV. Objection to the Claims

Claim 14 was objected to for not terminating with a period. Applicants thank the Examiner for noting the typographical error (which was not present in the original claims). Applicants' current listing of claims includes the period.

Claim 40 was objected to for the preposition "in." Applicants have amended the claim in accord with the Examiner's kind suggestion.

V. Rejections under 35 U.S.C. §112, first paragraph

Claims 1-25, 34-36, and 40 were rejected under 35 U.S.C. § 112, first paragraph, for lack of written description.

Claims 1-25, 34-36, and 40 were further rejected under 35 U.S.C. §112, first paragraph, allegedly because the specification does not enable any person skilled in the art to which it pertains, or with which it is most connected to make and use the invention commensurate in scope with the claims.

A. Written Description

The Examiner asserts that the specification fails to provide an adequate written description of the invention as claimed. The claims, as amended, are directed to a method for assaying an interaction between a test agent and a lipid-

bilayer-associated component. The method comprises providing a surface detector array device having one or more bilayer-compatible surface regions including a component associated with the lipid bilayer expanse. The device is contacted with a bulk aqueous phase comprising the test agent which binds to the lipid bilayer-associated component. The membrane fluidity of one or more of the lipid expanses is decreased by this binding. The membrane fluidity is evaluated to determine interaction of the test agent and the lipid bilayer-associated component.

1. Legal Standard for Written Description

According to M.P.E.P. § 2163.02, an objective standard for determining compliance with the written description requirement is, "does the description clearly allow persons of skill in the art to recognize that he or she invented what is claimed." *In re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1991). An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams and formulas that fully set forth the claimed invention. *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997).

Unlike the "enablement" requirement, the "written description" requirement of 35 U.S.C. §112, first paragraph is not concerned with support commensurate with the breadth of the claims. The essential purpose of the written description requirement is to show the possession of the invention as of the filing date as a *prima facie* date of invention. *In re Smith*, 481 F.2d 910, 178 U.S.P.Q. 620, 623 (CCPA 1973). Accordingly, the specification is required to contain a statement that adequately describes the invention as claimed. However, the invention need not be described in *ipsis verbis* in order to satisfy the description requirement. See *In re Lukach, Olson, and Spurlin*, 169 U.S.P.Q. 795, 796 (CCPA 1971).

"It is not required that the application describe the claim limitations in greater detail than the invention warrants. The description must be sufficiently clear that

persons of skill in the art will recognize that the applicant made the invention having those limitations." *Martin v. Mayer*, 823 F.2d 500, 3 USPQ2d 1333 (Fed. Cir. 1987).

2. Meeting the Legal Standard

As noted above, the present method comprises the steps of (i) providing a surface detector array device, (ii) contacting the device with a bulk aqueous phase comprising a test agent, whereby the membrane fluidity of the one or more lipid bilayer expanses is decreased when the test agent binds with the lipid bilayer-associated component, and (iii) evaluating the membrane fluidity of one or more of the lipid bilayer expanses.

With regard to step (i), surface detector array devices are described in paragraphs [0048]-[0065] and an exemplary device is shown in Fig. 1.

With regards to (ii), contacting the device with a bulk aqueous phase comprising a test agent and detecting the binding of the test agent to the lipid bilayer-associated component through their effects on the membrane fluidity of the lipid bilayer is described in paragraph [0070].

Evaluation of the membrane fluidity to detect binding of the test agent and the lipid bilayer-associated component are described in paragraph [0070]. Methods for measuring and evaluating the membrane fluidity are described in paragraph [0072].

The Examiner states that the claims are drawn to "a genus of test agents and lipid bilayer-associate components" (Office action dated September 19, 2005, page 4). However, those skilled in the art would recognize that the present method is not dependent upon the particular test agent. Rather it is the evaluation of the interaction of a test agent with a lipid bilayer-associated component through the effects of the interaction on the membrane fluidity of the lipid bilayer that is the subject of the present invention. Those skilled in the art would be well aware that the present method is useful regardless of the test agent selected.

The Examiner is further directed to the Declaration under 37 C.F.R. § 1.132 by Victoria Yamazaki (unsigned copy enclosed herewith, the executed copy will follow under separate cover) detailing the use of the presently claimed method with the anti-ICAM-1 antibody as the test agent.

In view of the teachings in the specification, the level of skill, and the knowledge in the art, one skilled in the art would reasonably conclude that Applicants were in possession of the claimed invention at the time the invention was filed.

B. Enablement

The first paragraph of 35 U.S.C. §112 requires that the specification of a patent enable any person skilled in the art to which it pertains to make and use the claimed invention without undue experimentation (e.g., *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

The enablement requirement is met if the description enables any mode of making and using the claimed invention (*Engel Industries, Inc. v. Lockformer Co.,* 946 F.2d 1528, 20 USPQ2d 1300 (Fed. Cir. 1991).

An invention is enabled even though the disclosure may require some routine experimentation to practice the invention. *Hybritech Inc,* V. *Monoclonal Antibodies, Inc.,* 802 F.2d 1367, 1384, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986). The fact that the required experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *MLT. v A.B. Fortia,* 774 F.2d 1104, 227 U.S.P.Q. 428 (Fed. Cir. 1985).

As noted above, the amended claims are directed to a method for assaying an interaction between a test agent and a lipid bilayer-associated component using a surface detector array device including a plurality of lipid bilayer expanses. When the test agent binds to the lipid bilayer-associated component, the membrane fluidity of the lipid bilayer expanse(s) is decreased. Example 7 of the present invention provides guidance for an exemplary test agent and lipid bilayer-

associated component, specifically the cholera toxin and the ganglioside GM1 membrane target.

The Examiner is further directed to the enclosed Declaration under 37 C.F.R. § 1.132 by Victoria Yamazaki. As noted in points 6-8 of the Declaration, the binding of the anti-ICAM-1 antibody (the test agent) to the ICAM-1 antigen protein (the lipid bilayer-associated component), which was displayed in the lipid membrane of a surface detector array, was detected by examining membrane fluidity using fluorescence recovery after photobleaching (FRAP) (Yamazaki *et al.*, *J Am Chem Soc*, 127, 2826-2827, 2005). As seen in the article, enclosed herewith, there is a direct correlation between the method for detecting the interaction of CTB and GM1 as in the present specification (and described in the article) and the interaction of anti-ICAM-1 antibody and the ICAM-1 antigen protein. The methods described in the present application were shown in the article to be useful generally for receptor-ligand biding.

As will be appreciated, the test agents and components used in the two experiments are dissimilar, yet, yield similar results for assaying the interaction. For example, the antibody is bivalent, as opposed to CTB which is polyvalent; the antigen is a protein, as opposed to GM1 which is lipid. This exemplifies the general use of the present method for a variety of unrelated test agents and lipid bilayer-associated components.

The Examiner further states that "[t]he prior art teaches that integral membrane proteins in supported bilayers may often be non-functional, and therefore incapable of interacting with test agents" (see page 9, lines 4-5 of the Office action mailed September 19, 2005). As described in the Boxer *et al.* reference, there is a possibility that the proximity of the tail of the integral membrane protein to the solid substrate may retard lateral motion and reduce the fluidity of the test agent in the plane of the lipid bilayer. However, those skilled in the art recognize that this proximity may be adjusted i.e. through insertion of a molecular cushion between the lipid bilayer and the solid substrate. For example, it

has been demonstrated that tethering lipid bilayers to the solid substrate with polyethylene glycol (PEG) considerably enhances the lateral mobility of integral membrane proteins in the lipid bilayer (see Wagner and Tamm (2000) *Biophys J.*, 79:1400-1414). This PEG "cushion" was shown to preserve the fluidity of a number of integral membrane proteins, including cytochrome b_5 and annexin V. Moreover, this system has been used to demonstrate the functionality of reconstituted SNAREs, integral membrane proteins critically important in subcellular vesicular fusion (see Wagner and Tamm (2001) *Biophys J.* 81:266-275). Those skilled in the art would readily recognize the applicability of the PEG "cushion" as well as others in the "suitable substrate" as presently claimed to enhance the lateral mobility of integral membrane proteins. Thus, those skilled in the art would not expect lipid bilayer-associated components to be non-functional based on this teaching in the referenced Boxer *et al.* article.

Accordingly, Applicants submit that the specification would enable any person skilled in the art to which it pertains to make and use the claimed invention.

In light of the above, Applicants submit that the present claims satisfy the requirements of 35 U.S.C. §112, first paragraph and respectfully request that the rejections be withdrawn.

VI. Rejection under 35 U.S.C. §112, second paragraph

Claims 1, 2, and 8-9 were rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. The Examiner had eight specific objections, which are set forth and discussed below.

1. The Examiner objected to claim 1 as allegedly incomplete for omitting essential steps. Claim 1 is amended to recite all the necessary steps involved in the method.

2. The Examiner objected to claim 1 as allegedly unclear whether the plurality of lipid bilayer expanses are reciting further components of the surface detector array device. The Examiner is directed to Fig. 1, where it is clear that the plurality of lipid bilayer expanses localized above the plurality of distinct bilayer-compatible surface regions are components of the surface detector array device. Accordingly, Applicants submit that the language is clear in view of the teaching in the specification.

3. Claims 9-13 were objected to for the language "membrane fluidity" and "acyl chain mobility" as allegedly unclear. Applicants' amendments obviate this objection.

4. Claims 17-18 were objected to for the language "membrane appearance" as allegedly not defined. Applicants' amendments obviate this objection.

5. Claim 36 was objected to for the language "cell-vesicle" as allegedly unclear. Applicants submit that this term is recognized in the art as referring to an empty cell, usually erythrocyte. Applicants enclose pages 489-491 of a standard textbook referencing "ghost cells" which are synonymous with "cell-vesicles" (Molecular Biology of the Cell, third edition, Alberts *et al.* eds., Garland Publishing, Inc., New York, 1994).

6. Claim 36 was further objected to for the language "phantom cell" as allegedly not defined. Applicants respectfully submit that the term "phantom cell" would be recognized by one skilled in the art to refer to an empty cell, usually erythrocyte. This term is routinely used in the art as evidenced by a PubMed search for "phantom cell" that returned 363 articles with the term. Applicants further enclose an abstract of an article by Sackmann, referenced in the present specification at page 3, lines 9-10 describing the use of phantom cells. Finally, Applicants enclose pages 489-491 of the Molecular Biology of the Cell textbook (noted above in point 5) referencing "ghost cells" which are synonymous with "phantom cells."

7. Claim 36 was additionally objected to for the language "giant" as a relative term that allegedly renders the claim indefinite. Applicants respectfully submit that the term "giant liposome" is standard in the art and is understood to refer to liposomes with a diameter greater than large unilamellar liposomes or about 10 μm or greater. Applicants refer the Examiner to an article (Riquelme, *et al.*, Biochemistry, 29(51):11215-11222, 1990) published in 1990 describing the use of giant liposomes (abstract enclosed).

8. Claim 40 was objected to for the language "the interaction of the test agent with the lipid bilayer-associated component" for allegedly insufficient antecedent basis. Claim 40 as amended provides proper antecedent basis for this language.

In light of the above amendments, the teaching in the specification, and/or the knowledge of those skilled in the art, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. §112, second paragraph.

VII. Rejections under 35 U.S.C. §102

Claims 1-2, 4-8, 14-15, 19-20, and 34-36 were rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Boxer *et al.* (U.S. Patent No. 6,228,326).

Applicants respectfully traverse these rejections.

A. The Present Invention

The present method as embodied by claim 1 comprises a method for assaying an interaction between a test agent and a lipid bilayer-associated component. The method comprises (i) providing a surface detector array device comprising (a) a substrate having a surface defining a plurality of distinct bilayer-compatible surface regions separated by one or more bilayer barrier regions, (b) a plurality of lipid bilayer expanses localized above the plurality of distinct bilayer-compatible surface regions, the lipid bilayer expanses having a component associated with the lipid bilayer expanse; (iii) contacting the device with a bulk

aqueous phase comprising a test agent that specifically binds to the lipid bilayer-associated component, whereby the membrane fluidity of at least one of the plurality of lipid bilayer expanses decreases when said test agent binds to said lipid bilayer-associated component; and (iv) evaluating the fluidity of one or more of the lipid bilayer expanses.

B. The Cited References

BOXER ET AL. relate to a surface detector array device, comprising (i) a substrate having a surface defining a plurality of distinct bilayer-compatible surface regions separated by one or more bilayer barrier regions, (ii) a lipid bilayer expanse stably localized on each of said bilayer-compatible surface regions, (iii) an aqueous film interposed between each bilayer-compatible surface region and corresponding lipid bilayer expanse, wherein each lipid bilayer expanse is stably localized above each bilayer-compatible surface, and (iv) a bulk aqueous phase covering the lipid bilayer expanses.

C. Analysis

According to the M.P.E.P. § 2131, "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference".

Boxer *et al.* fail to teach at least contacting the device with a test agent that specifically binds to a lipid bilayer-associated component, whereby the membrane fluidity of at least one of the plurality of lipid bilayer expanses decreases and evaluating the membrane fluidity of one or more of the lipid bilayer expanses. Instead, Boxer *et al.* disclose evaluating binding with transmembrane voltage and current.

Accordingly, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 102(b).

VIII. Rejections under 35 U.S.C. §103

Claim 3 was rejected under 35 U.S.C. §103 as allegedly obvious over Boxer *et al.* (the '326 patent), or, alternatively, Boxer *et al.* (PCT Publication No. WO 98/23948) in view of Gutsmann *et al.* (Biophysical Journal, 80:2935-2945, 2001).

Claims 9-10 were rejected under 35 U.S.C. §103 as allegedly obvious over Boxer *et al.* (the '326 patent), or, alternatively, Boxer *et al.* (the '948 publication) in view of Groves *et al.* (Science, 275:651-653, 1997).

Claims 11-12 were rejected under 35 U.S.C. §103 as allegedly obvious over Boxer *et al.* (the '326 patent), or, alternatively, Boxer *et al.* (PCT Publication No. WO 98/23948) in view of Swamy *et al.* (Biochemistry, 36:7403-7407, 1997).

Claims 11 and 13 were rejected under 35 U.S.C. §103 as allegedly obvious over Boxer *et al.* (the '326 patent), or, alternatively, Boxer *et al.* (PCT Publication No. WO 98/23948) in view of Rooney *et al.* (J. Biol. Chem., 259:8281-8285, 1984).

Claims 17-18 were rejected under 35 U.S.C. §103 as allegedly obvious over Boxer *et al.* (the '326 patent), or, alternatively, Boxer *et al.* (PCT Publication No. WO 98/23948) in view of Rinia *et al.* (Biochemistry, 39:5852-5858, 2000).

Claims 22-23 were rejected under 35 U.S.C. §103 as allegedly obvious over Boxer *et al.* (the '326 patent), or, alternatively, Boxer *et al.* (PCT Publication No. WO 98/23948) in view of Yang *et al.* (J. Mol. Biol., 229:286-290, 1993).

Claims 24-25 were rejected under 35 U.S.C. §103 as allegedly obvious over Boxer *et al.* (the '326 patent), or, alternatively, Boxer *et al.* (PCT Publication No. WO 98/23948) in view of Hirn *et al.* (Biophysical J., 77:2066-2074, 1999).

Claim 40 was rejected under 35 U.S.C. §103 as allegedly obvious over Boxer *et al.* (the '326 patent), or, alternatively, Boxer *et al.* (PCT Publication No. WO 98/23948) in view of Keinanen *et al.* (U.S. Patent No. 6,235,535).

Claims 11-25 are canceled. Applicants address the rejection of the remaining claims below.

These rejections are respectfully traversed.

A. The Present Invention is described above.

B. The Cited References

BOXER ET AL., THE '326 PATENT is described above.

BOXER ET AL., THE '948 PUBLICATION corresponds to the '326 patent for purposes of the Examiner's rejections and is thus, not separately discussed.

GUTSMANN ET AL. investigate the relationship between the interaction of CAP-18-derived peptides with membranes and the activity of the peptides.

GROVES ET AL. describe partition supported lipid bilayers on arrays. The lipid bilayers are confined by the barriers. The bilayers retain lateral fluidity within the "corrals."

KEINANEN ET AL. relate to a fluorescence-based immunoassay method for the detection of an analyte. The method includes attaching receptor molecules to a lipid membrane, contacting a sample with the receptor molecules, and measuring the fluorescence change caused by a change of aggregation level of the receptor molecules.

C. Analysis

According to the M.P.E.P. § 2143, "to establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Third, the prior art references (or references when combined) must teach or suggest all the claim limitations."

1. Rejection of claim 3

As noted above, the '326 patent fails to teach at least contacting the device with a test agent that specifically binds to a lipid bilayer-associated component,

whereby the membrane fluidity of at least one of the plurality of lipid bilayer expanses decreases and evaluating the membrane fluidity of one or more of the lipid bilayer expanses. Gutsmann *et al.* use several biophysical techniques to investigate the interaction of CAP-18 peptides and a membrane. As described by Gutsmann *et al.*, the peptides bind to and/or intercalate into the membrane. Importantly, Gutsmann *et al.* does not teach a method including contacting a membrane with a test agent that binds to the lipid bilayer-associated component. Further, Gutsmann *et al.* make no mention of evaluating the fluidity of the membrane.

2. Rejection of claims 9-10

The '326 patent discloses that binding of a test agent to a lipid bilayer-associated component can be detected by monitoring a physical property of the membrane. The physical properties described in the '326 patent are transmembrane voltage and current. However, the voltage and current described in the original patent is unique to the protein involved (e.g. acetylcholine receptor functions as a ligand-gated ion channel) and not the membrane itself. Hence, upon binding of acetylcholine to its receptor, the receptor protein itself allows ions to pass through, functioning like a pore in the membrane. It is NOT the membrane itself that functions as the pore.

In contrast, in the presently amended claims, the device is contacted with a bulk aqueous phase comprising a test agent that specifically binds to the lipid bilayer-associated component, whereby the membrane fluidity of at least one of the plurality of lipid bilayer expanses decreases when said test agent binds to said lipid bilayer-associated component. The fluidity of the one or more lipid bilayer expanses is then evaluated.

The teaching in Groves *et al.* does not make up for the deficiency in the '326 patent. Groves *et al.* teach an array similar to that described in the '326 patent in that lipid bilayers are supported on an array such that bilayer expanses are

separated by barriers. Groves *et al.* further teach that spatially addressable molecular libraries can be synthesized and displayed on the surface of the bilayer expanses. Nowhere does Groves *et al.* make any mention of (at least) contacting the device with a bulk aqueous phase comprising a test agent that specifically binds to the lipid bilayer-associated component, whereby the membrane fluidity of at least one of the plurality of lipid bilayer expanses decreases when the test agent binds to the lipid bilayer-associated component.

3. Rejection of claim 40

The '326 patent teaches evaluating transmembrane voltage and current, which are unique to the protein involved (e.g. the acetylcholine receptor functions as a ligand-gated ion channel) and not the membrane itself. Upon binding of acetylcholine to its receptor, the receptor protein allows ions to pass through, functioning like a pore in the membrane. It is NOT the membrane itself that functions as the pore and not a physical property of the membrane that is evaluated. The '326 patent further teaches measuring membrane fluidity solely as a means for evaluating the physiological qualities of the lipid bilayer. Fluidity was never mentioned or tested with respect to evaluation of binding of a test agent to a lipid bilayer-associated component.

Nor does Keinanen *et al.* supply the missing teaching. Instead, Keinanen *et al.* teach detecting analyte-receptor binding by fluorescence changes by aggregation of the receptors when bound to a multivalent analyte.

As the references, alone or in combination, fail to teach or suggest all the claim limitations, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 103.

IX. Obviousness-Type Double Patenting Rejections

Claims 1-25, 34-36 and 40 were rejected under the judicially created doctrine of obviousness-type double patenting as being directed to an invention not patentably

distinct from claim 22 of co-owned patent no. 6,699,719 in view of Boxer *et al.*, the '326 patent.

A. The Present Claims

The present method as embodied by claim 1 comprises a method for assaying an interaction between a test agent and a lipid bilayer-associated component. The method comprises (i) providing a surface detector array device comprising (a) a substrate having a surface defining a plurality of distinct bilayer-compatible surface regions separated by one or more bilayer barrier regions, (b) a plurality of lipid bilayer expanses localized above the plurality of distinct bilayer-compatible surface regions, the lipid bilayer expanses having a component associated with the lipid bilayer expanse; (iii) contacting the device with a bulk aqueous phase comprising a test agent that specifically binds to the lipid bilayer-associated component, whereby the membrane fluidity of at least one of the plurality of lipid bilayer expanses decreases when said test agent binds to said lipid bilayer-associated component; and (iv) evaluating the fluidity of one or more of the lipid bilayer expanses.

B. U.S. Patent No. 6,699,719 (the '719 patent)

The '719 patent relates to a multiplexed assay, comprising the steps of:

(i) providing a surface detection array device, said device comprising a substrate having a surface defining a plurality of distinct bilayer-compatible surface regions separated by one or more bilayer barrier regions, said bilayer-compatible surface regions and said bilayer barrier regions being formed of different materials, a first lipid bilayer expanse having a first composition and stably localized above a first of said plurality of distinct bilayer-compatible surface regions, a second lipid bilayer expanse having a second composition different from said first composition and stably localized above a second of said plurality of distinct bilayer-compatible surface regions, wherein each of said expanses is localized above each of said

surface regions in the absence of covalent linkages between each of said lipid bilayer expanses and each of said bilayer-compatible surface regions, and is separated therefrom by an aqueous film interposed between said bilayer-compatible surface regions and said corresponding lipid bilayer expanses;

(ii) contacting said device with a bulk aqueous phase comprising a test agent; and

(iii) assaying an interaction between said test agent and said first composition and an interaction between said test agent and said second composition.

C. Analysis

The purpose of obviousness-type double patenting is to prevent improper timewise extension of patent rights by prohibiting the issuance of claims in a second application which are not "patentably distinct" from the claims of the first patent. M.P.E.P. 804(II)(B)

The present method requires a step of evaluating the membrane fluidity of one or more of the lipid bilayer expanses to assay a binding interaction between a test agent and a lipid bilayer-associated component. Thus, the present method evaluates a membrane property, namely membrane fluidity, in order to assay the interaction of the test agent and the lipid bilayer-associated component. This feature is not an obvious variation of the "assaying an interaction between said test agent and said first composition and an interaction between said test agent and said second composition."

Accordingly, Applicants respectfully request withdrawal of the obviousness-type double patenting rejections.

X. Conclusion

Applicants respectfully submit that the pending claims are in condition for immediate allowance. The undersigned invites the Examiner to call (650) 838-

4410 with any questions or comments. The Commissioner is hereby authorized and requested to charge any deficiency in fees herein to Deposit Account No. 50-2207.

Respectfully submitted,
Perkins Coie LLP

Date: Jan. 20, 2006

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☐ 1: Biochemistry. 1990 Dec 25;29(51):11215-22.

Giant liposomes: a model system in which to obtain patch-clamp recordings of ionic channels.

Riquelme G, Lopez E, Garcia-Segura LM, Ferragut JA, Gonzalez-Ros JM.

Department of Neurochemistry, University of Alicante, Spain.

Cell-size, giant liposomes have been formed by submitting a mixture of asolectin lipid vesicles and native membranes from Torpedo, highly enriched in acetylcholine receptor (AcChR), to a partial dehydration/rehydration cycle [Criado, M., & Keller, B. U. (1987) FEBS Lett. 224, 172-176]. Giant liposomes can be prepared in bulk quantities, in the absence of potentially damaging detergents or organic solvents, and their formation is mediated by membrane fusion phenomena. In fact, fluorescence microscopy and freeze-fracture data indicate that protein and lipid components of the initial membranes and lipid vesicles are homogenously distributed in the resulting liposomes. Giant liposomes containing AcChR have been used as a model to evaluate whether this system can be used to monitor the activity of ionic channels by using high-resolution, patch-clamp techniques. Excised liposome patches in an "inside-out" configuration have been used in this work. We find that the most frequent pattern of electrical activity in response to the presence of acetylcholine in the patch pipet corresponds to a cation-specific channel exhibiting a dominant conductance level and a sublevel of approximately 78 and 25 pS, respectively. Such channel activity exhibits the pharmacological specificity, ion channel activation, ion selectivity, and desensitization properties expected from native Torpedo AcChR. Thus, it appears that the giant liposome technique offers a distinct advantage over other reconstitution procedures in that it provides a unique opportunity to undertake simultaneous biochemical, morphological, and electrophysiological studies of the incorporated ionic channel proteins.

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☐ 1: Science. 1996 Jan 5;271(5245):43-8.

Supported membranes: scientific and practical applications.

Sackmann E.

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Scientific and practical applications of supported lipid-protein bilayers are described. Membranes can be covalently coupled to or separated from solids by ultrathin layers of water or soft polymer cushions. The latter systems maintain the structural and dynamic properties of free bilayers, forming a class of models of biomembranes that allow the application of a manifold of surface-sensitive techniques. They form versatile models of low-dimensionality complex fluids, which can be used to study interfacial forces and wetting phenomena, and enable the design of phantom cells to explore the interplay of lock-and-key forces (such as receptor-ligand binding) and universal forces for cell adhesion. Practical applications are the design of (highly selective) receptor surfaces of biosensors on electrooptical devices or the biofunctionalization of inorganic solids.

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THE CELL
THIRD EDITION

Bruce Alberts • Dennis Bray
Julian Lewis • Martin Raff • Keith Roberts
James D. Watson

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Front cover: The photograph shows a rat nerve cell in culture. It is labeled (*yellow*) with a fluorescent antibody that stains its cell body and dendritic processes. Nerve terminals (*green*) from other neurons (not visible), which have made synapses on the cell, are labeled with a different antibody. (Courtesy of Olaf Mundigl and Pietro de Camilli.)

Dedication page: Gavin Borden, late president of Garland Publishing, weathered in during his mid-1980s climb near Mount McKinley with MBoC author Bruce Alberts and famous mountaineer guide Mugs Stump (1940–1992).

Back cover: The authors, in alphabetical order, crossing Abbey Road in London on their way to lunch. Much of this third edition was written in a house just around the corner. (Photograph by Richard Olivier.)

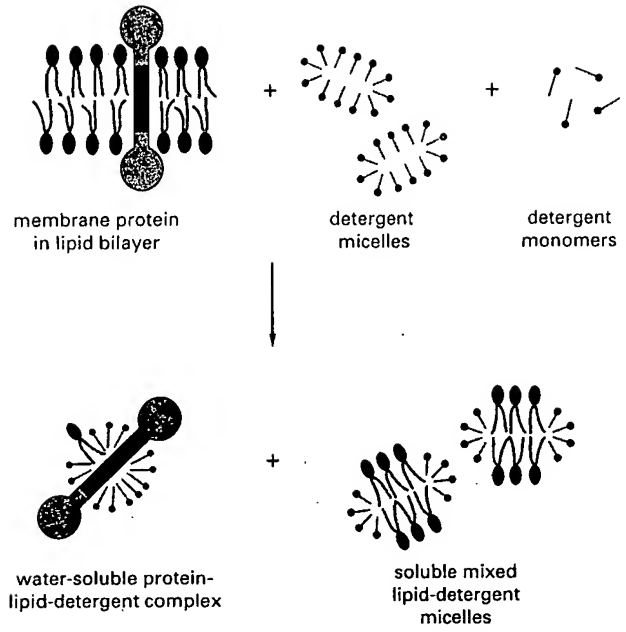

Figure 10–19 Solubilizing membrane proteins with a mild detergent. The detergent disrupts the lipid bilayer and brings the proteins into solution as protein-lipid-detergent complexes. The phospholipids in the membrane are also solubilized by the detergent.

biochemist are **detergents,** which are small amphipathic molecules that tend to form micelles in water (Figure 10–18). When mixed with membranes, the hydrophobic ends of detergents bind to the hydrophobic regions of the membrane proteins, thereby displacing the lipid molecules. Since the other end of the detergent molecule is polar, this binding tends to bring the membrane proteins into solution as detergent-protein complexes (although some tightly bound lipid molecules may also remain) (Figure 10–19). The polar (hydrophilic) ends of detergents can be either charged (ionic), as in the case of *sodium dodecyl sulfate* (SDS), or uncharged (nonionic), as in the case of the *Triton* detergents. The structures of these two commonly used detergents are illustrated in Figure 10–20.

With strong ionic detergents, such as SDS, even the most hydrophobic membrane proteins can be solubilized. This allows them to be analyzed by *SDS polyacrylamide-gel electrophoresis* (discussed in Chapter 4), a procedure that has revolutionized the study of membrane proteins. Such strong detergents unfold (denature) proteins by binding to their internal "hydrophobic cores," thereby hindering the proteins inactive and unusable for functional studies. Nonetheless, proteins can be readily purified in their SDS-denatured form, and in some cases the purified protein can be renatured, with recovery of functional activity, by removing the detergent.

Many hydrophobic membrane proteins can be solubilized and then purified in an active, if not entirely normal, form by the use of mild detergents, such as Triton X-100, that bind to the membrane-spanning segments of the protein. In this way functionally active membrane protein systems can be reconstituted from purified components, providing a powerful means to analyze their activities (Figure 10–21).

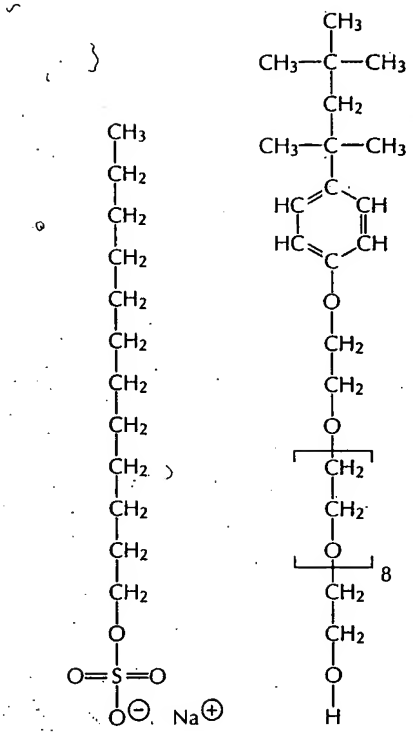

Figure 10–20 The structures of two commonly used detergents. Sodium dodecyl sulfate (SDS) is an anionic detergent, and Triton X-100 is a nonionic detergent. The hydrophobic portion of each detergent is shown in *green*, and the hydrophilic portion is shown in *blue*. Note that the bracketed portion of Triton X-100 is repeated about eight times.

The Cytoplasmic Side of Membrane Proteins Can Be Readily Studied in Red Blood Cell Ghosts [10]

More is known about the plasma membrane of the human red blood cell (Figure 10–22) than about any other eucaryotic membrane. There are a number of reasons for this. Red blood cells are available in large numbers (from blood banks, for example) relatively uncontaminated by other cell types. Since they have no nucleus or internal organelles, the plasma membrane is their only membrane, and it can be isolated without contamination by internal membranes (thus avoiding a serious problem encountered in plasma membrane preparations from other

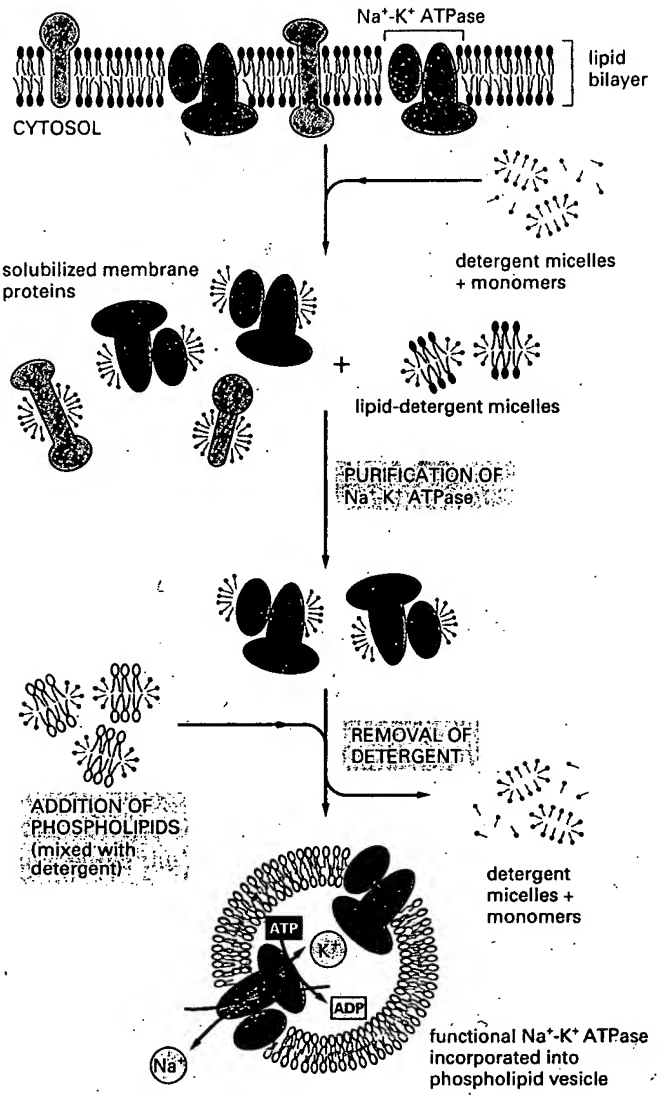

Figure 10–21 The use of mild detergents for solubilizing, purifying, and reconstituting functional membrane protein systems. In this example functional Na+-K+ ATPase molecules are purified and incorporated into phospholipid vesicles. The Na+-K+ ATPase is an ion pump that is present in the plasma membrane of most animal cells; it uses the energy of ATP hydrolysis to pump Na^+ out of the cell and K^+ in, as discussed in Chapter 11.

cell types in which the plasma membrane typically constitutes less than 5% of the cell's membrane). It is easy to prepare empty red blood cell membranes, or "ghosts," by putting the cells in a medium with a lower salt concentration than the cell interior. Water then flows into the red cells, causing them to swell and burst (lyse) and release their hemoglobin (the major nonmembrane protein). Membrane ghosts can be studied while they are still leaky (in which case any reagent can interact with molecules on both faces of the membrane), or they can

Figure 10–22 A scanning electron micrograph of human red blood cells. The cells have a biconcave shape and lack nuclei. (Courtesy of Bernadette Chailley.)

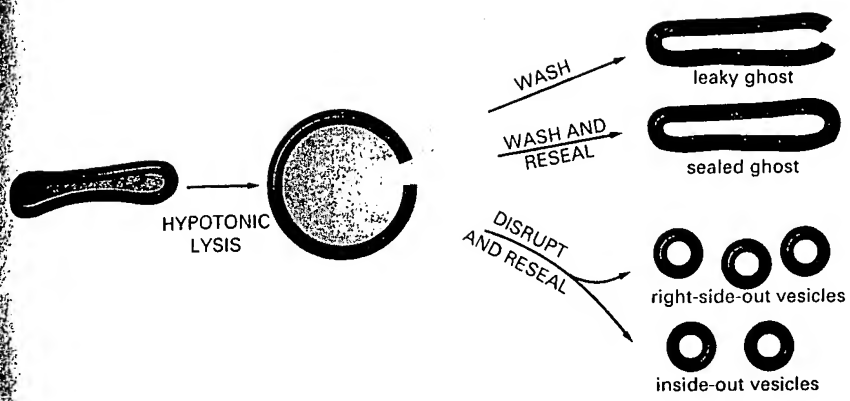

Figure 10–23 The preparation of sealed and unsealed red blood cell ghosts and of right-side-out and inside-out vesicles. As indicated, the red cells tend to rupture in only one place, giving rise to ghosts with a single hole in them. The smaller vesicles are produced by mechanically disrupting the ghosts; the orientation of the membrane in these vesicles can be either right-side-out or inside-out, depending on the ionic conditions used during the disruption procedure.

be allowed to reseal so that water-soluble reagents cannot reach the internal face. Moreover, since sealed *inside-out* vesicles can also be prepared from red blood cell ghosts (Figure 10–23), the external side and internal (cytoplasmic) side of the membrane can be studied separately. The use of sealed and unsealed red cell ghosts led to the first demonstration that some membrane proteins extend across the lipid bilayer (discussed below) and that the lipid compositions of the two halves of the bilayer are different. Like most of the basic principles initially demonstrated in red blood cell membranes, these findings were later extended to the membranes of nucleated cells.

The "sidedness" of a membrane protein can be determined in several ways. One is to use a covalent labeling reagent (for example, one carrying a radioactive or fluorescent marker) that is water soluble and therefore cannot penetrate the lipid bilayer; such a marker will attach covalently only to the portion of the protein on the exposed side of the membrane. The membranes are then solubilized with detergent and the proteins separated by SDS polyacrylamide-gel electrophoresis. The labeled proteins can be detected either by their radioactivity (by autoradiography of the gel) or by their fluorescence (by exposing the gel to ultraviolet light). By using such *vectorial labeling*, it is possible to determine how a particular protein, detected as a band on a gel, is oriented in the membrane: for example, if it is labeled from both the external side (when intact cells or sealed ghosts are labeled) and the internal (cytoplasmic) side (when sealed inside-out vesicles are labeled), then it must be a transmembrane protein. An alternative approach is to expose either the external or internal surface to membrane-impermeant proteolytic enzymes: if a protein is partially digested from both surfaces, it must be a transmembrane protein. In addition, labeled antibodies that bind only to one part of a protein can be used to determine if that part of a transmembrane protein is exposed on one side of the membrane or the other.

When the plasma membrane proteins of the human red blood cell are studied by SDS polyacrylamide-gel electrophoresis, approximately 15 major protein bands are detected, varying in molecular weight from 15,000 to 250,000. Three of these proteins—*spectrin*, *glycophorin*, and *band 3*—account for more than 60% (by weight) of the total membrane protein (Figure 10–24). Each of these proteins is arranged in the membrane in a different manner. We shall, therefore, use them as examples of three major ways that proteins are associated with membranes, not only in red blood cells, but in other cells as well.

Spectrin Is a Cytoskeletal Protein Noncovalently Associated with the Cytoplasmic Side of the Red Blood Cell Membrane [11]

Most of the protein molecules associated with the human red blood cell membrane are peripheral membrane proteins associated with the cytoplasmic side of the lipid bilayer. The most abundant of these proteins is **spectrin,** a long, thin,

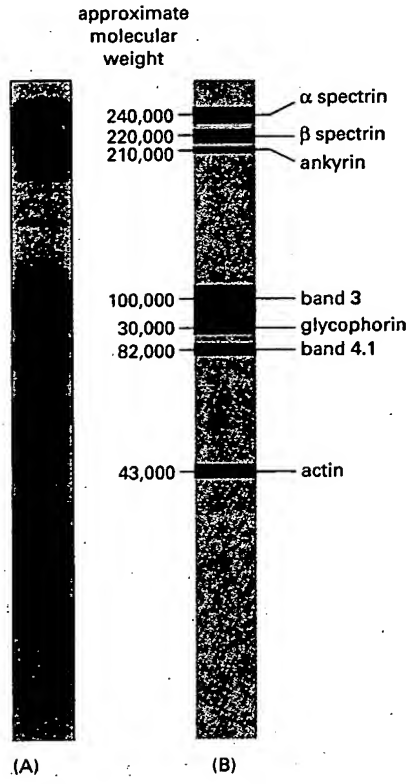

Figure 10–24 SDS polyacrylamide-gel electrophoresis pattern of the proteins in the human red blood cell membrane. The gel in (A) is stained with Coomassie blue. The positions of some of the major proteins in the gel are indicated in the drawing in (B); glycophorin is shown in *red* to distinguish it from band 3. Other bands in the gel are omitted from the drawing. The large amount of carbohydrate in glycophorin molecules slows their migration so that they run almost as slowly as the much larger band 3 molecules. (A, courtesy of Ted Steck.)